Reida Akam's Lab Notebook

June 1, 2015

Objectives:

- Perform genetic transformation of pGLO into E.Coli cells
- Practice lab skills & sterile techniques, especially for the iGEM members that have not been acquainted to Bio Lab

Results

- pGLO Transformation from yesterday did not work. It may be due to the fact that we used old plates, and therefore we need to make new ones

June 2, 2015

Objectives:

- Learn about lab preparation (autoclaving, turning on the water bath, sterilizing the hood)
- Made Plates:
    - LB
    - LB + Amp
    - LB + Amp + Ara
    - LB + Amp + Ara + Glucose
- Successfully rehydrate plasmids from the kit and transform using the iGEM protocol and dH5 α

Procedures

- Reida performed the first transformation using iGEM parts which included the RFP control in the efficiency kit, and the same part located on a rehydrated pSB1A10 backbone. She used the standard iGEM Transformation Protocol, but made a few adjustments; instead of SOC, LB was used and instead of a large shaking incubator, a Thermoshaker was used. The plates used from all eight transformations contained glucose.

Results

- Plasmids used were not viable - but this is very unlikely. In order to overcome this, we will do:
    - Transformation with the plates we made today and original pGLO
    - Transformation with a set of new plates and not original pGLO
    - Transformation with a set of new plates and the original pGLO
- All of the RFP control plasmids from the transformation efficiency kit did not transform, while there was an average of 3 colonies grown from the plate kit plasmid, probably due to the incorrect concentration of agar on the plates.

June 3, 2015

Objectives:

- Prepare rehydrated plasmids from the kit to successfully result in fluorescent colonies

Procedures:

- Because of the lack of chloramphenicol antibiotic in the lab, the next transformation was of the RFP plasmid from the day before, and three other types of CFP. To check the efficiency of HB101 cells during transformation, Reida replated the RFP using the HB101 and the CFP with dH5α cells. Again, the standard iGEM Transformation Protocol was used but with LB broth instead of SOC and a shaking incubator.

Results:

- While all of the CFP plates had plenty of colonies, the RFP was unsuccessful which leads us to believe that our competent cells are not viable for transformation with plasmids from the plate kit.

June 4, 2015

Objectives:

- Make chloramphenicol plates with different concentrations
- Inoculate RFP and CFP colonies for future miniprep

Procedures:

- Christina made different concentrations of chloramphenicol stock solutions and then made plates containing the chloramphenicol with the different concentrations:
  - 12.5 mg/ml Chloramphenicol Stock Solution
  - 25 mg/ml Chloramphenicol Stock Solution
  - 34 mg/ml Chloramphenicol Stock Solution
  - 50 mg/ml Chloramphenicol Stock Solution
- Reida inoculated 2 colonies from each plate of RFP and CFP for miniprep for tomorrow in LB broth and glucose for the RFP which were grown on a glucose rich plate.

Results:

- Because the RFP plasmid is modified, colonies should be colored red, as should the culture, but that was not the case. This is due to the inconsistency of the plasmid used for the transformation.
- Encountered some difficulties while making the plates. The LB agar solidified before we finished plating, we had to reheat it and add more arabinose/chloramphenicol afterwards.
- Our instructor advised us to only make 25mg/ml and we don't need to make the rest because 25mg/ml is usually the standard concentration.

June 5, 2015

Objectives:

- Perform miniprep on the RFP and CFP amplified bacteria

Procedures:

- For this first miniprep, we call it a lab skill workshop, so everyone was involved in purifying the plasmids; we had Xiao Yue, Spencer, Zhang Zhan, So High, Reida, and Christina working on different samples. We followed the BioRad Miniprep Protocol.

Results:

Sample #	BioBrick	Plate	Well	Content	Plate Content	NA Concentration	Unit	260/280	Date
1	BBa_J04451	3755	2F	Generator: RFP	4ml LB/amp/glu RFP 10ul #1	87.6	ng/µl	2	6/5/2015
2	BBa_J04452	3755	2F	Generator: RFP	2ml LB/amp/glu RFP 10ul #2	67.8	ng/µl	1.93	6/5/2015
3	BBa_J04454	3755	2F	Generator: RFP	2ml LB/amp/glu RFP 100ul #1	31.3	ng/µl	2.01	6/5/2015
4	BBa_J04453	3755	2F	Generator: RFP	4ml LB/amp/glu RFP 100ul #2	60.6	ng/µl	1.89	6/5/2015
5	BBa_J04450	3755	2F	Generator: RFP	5ml LB/amp RFP 100ul	124.7	ng/µl	1.91	6/5/2015
9	BBa_I13600	3755	23F	Generator: CFP	4ml LB/amp 23F 200ul #1	42.5	ng/µl	1.93	6/5/2015
10	BBa_I13600	3755	23F	Generator: CFP	2ml LB/amp 23F 200ul #2	29.6	ng/µl	1.98	6/5/2015
7	BBa_J04456	3755	15J	Generator: CFP	2ml LB/amp 15J 200ul #1	53	ng/µl	1.89	6/5/2015
8	BBa_J04457	3755	15J	Generator: CFP	4ml LB/amp 15J 200ul #2	48.4	ng/µl	1.76	6/5/2015
6	BBa_J04455	3755	11J	Generator: CFP	5ml LB/amp 11J 200ul	56.4	ng/µl	1.97	6/5/2015

June 10, 2015

Objectives:

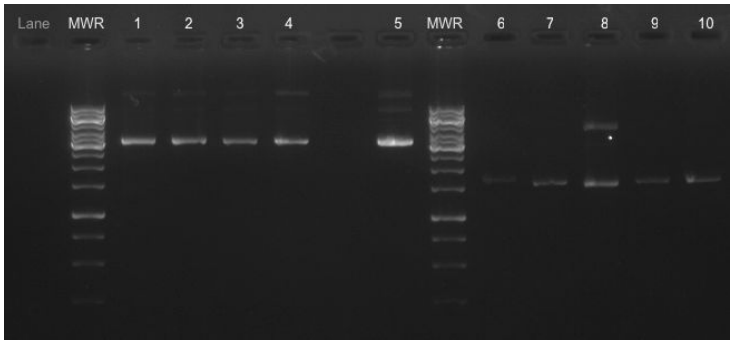
- Perform a gel electrophoresis of purified plasmids and perform a gel extraction

Procedures:

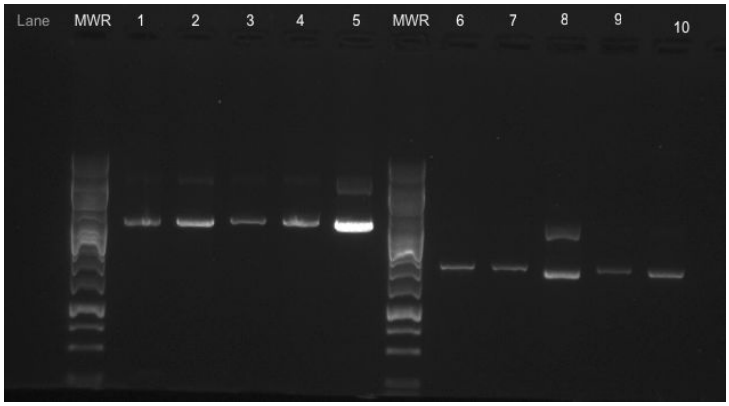
- Reida, Ann, and Christina all made agarose gel, but all at different concentration; Ann made a 1% gel, Reida .7%, Christina .4%. We used the TIANgel Midi DNA Purification Kit Protocol #1.

Results:

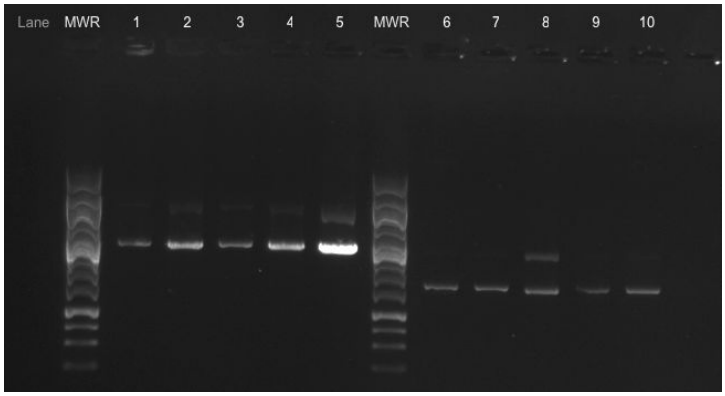
Ann Gel 1% agarose



Reida Gel .7% agarose



Christina Gel .4% agarose



Gel Extraction Results:

June 11th 2015

Objectives:

- Transform rehydrated luciferase plasmids from plate kit

Procedures:

- In addition to helping Christina make more agar plates, Reida transformed green firefly luciferase and orange firefly luciferase. Following the transformation procedures, the recovery time and transformation liquid amount were tested for optimization.

Results:

- No colonies from the orange firefly luciferase or with the green luciferase when plated on LB/chloramphenicol/arabinose plates for 40 min during the recovery period. This leads us to using the protocols that call for a 2 hour recovery period.

June 15th 2015

Objectives:

- Transform chromoprotein parts for restriction digest and ligation
- Inoculation of green luciferase with LB and SOC

- Make SOC media

Procedure:

- Used standard transformation protocol and inoculate luciferase using SOC media

Results:

Plate	Well	Part	Content	Backbone	Results
3753	3F	BBa_K880005	p/rbs	pSB1C3	colonies present
3754	16E	BBa_K089005	p/rbs2	pSB1C3	colonies present
3752	19E	BBa_K592009	blue chromo	pSB1C3	colonies present
3755	2I	BBa_K592011	green chromo	pSB1C3	colonies present
3756	5F	BBa_K1467201	gg blue chromo	pSB1C3	colonies present
3755	6K	BBa_K1033910	yellow chromo	pSB1C3	colonies present
3755	6M	BBa_K103396	lime chromo	pSB1C3	colonies present
3755	1D	BBa_B0024	terminator	pSB1A2	no colonies present
Plate 4	13L	BBa_E0040	GFP w/ amp	pSB1A2	colonies present
Plate 2	4N	BBa_E0020	CFP w/ chlor	pSB1C3	colonies present

- There were colonies for all transformations except from the terminator 1D probably due to transforming with low efficiency HB101 cells. The blue chromoprotein was found to be the wrong part. Instead of the plasmid being taken from plate 3752, the well was rehydrated from plate 3753, some sort of yeast plasmid.
- The inoculation went well and was followed up with a miniprep

June 16th 2015

Objectives:

- Do miniprep of inoculated luciferase plasmids using BioRad Kit
- Inoculate transformed chromoprotein plasmids
- Transform different terminator from plate kit, plasmid BBa_B0010 well: 16G plate: 3755

Procedures:

- Use standard transformation protocol
- Use TIAN gel kit for miniprep of luciferase
- Use SOC media for inoculation of chromoprotein parts

Results:

- The terminator also did not transform, the reason unknown
- The inoculation went well, miniprep for the chromoprotein parts will go on as planned

Green Luciferase MiniPrep Results

#	Date and Time	Nucleic Acid Conc.	Unit	260/280
1	6/16/2015 16:51:00	32.4	ng/µl	1.9
2	6/16/2015 16:52:00	38.5	ng/µl	1.88
3	6/16/2015 16:53:00	100.8	ng/µl	1.85
4	6/16/2015 16:54:00	32.1	ng/µl	1.91

June 17th 2015

Objectives:

- Miniprep chromoprotein parts using TIAN gel kit
- Re-Inoculation of chromoprotein parts 6K, 6M, 4N, 13L due to low miniprep yield
- Transformation of new terminator BBa_B0011 well: 1B plate:3755

Results:

- Because the 260/280 ratio was too high for some of the miniprepped samples, we inoculated more colonies from the specified plates to be miniprepped the next day
- The terminator finally successfully transformed

Chromoprotein Miniprep

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	260/280	Name
1	3F_K880005_1	6/17/2015 14:24:00	43.8	ng/µl	1.92	p/rbs
2	3F_K880005_2	6/17/2015 14:29:00	22	ng/µl	1.92	p/rbs
3	16E_K081005_1	6/17/2015 14:34:00	17.3	ng/µl	1.84	p/rbs2
4	16E_K081005_2	6/17/2015 14:36:00	17.3	ng/µl	1.87	p/rbs2
5	19E_K592009_1	6/17/2015 14:39:00	23.4	ng/µl	2.07	blue chromoprotein
6	19E_K592009_2	6/17/2015 14:40:00	18.5	ng/µl	1.98	blue chromoprotein
7	2I_K592011_1	6/17/2015 14:43:00	23.5	ng/µl	2	green chromoprotein
8	2I_K592011_2	6/17/2015 14:45:00	25.1	ng/µl	1.97	green chromoprotein
9	5F_K1467201_1	6/17/2015 14:47:00	16.8	ng/µl	1.98	gg blue chromoprotein
10	5F_K1467201_2	6/17/2015 14:49:00	19.1	ng/µl	1.91	gg blue chromoprotein
11	6K_K1033910_1	6/17/2015 14:51:00	14.6	ng/µl	1.93	yellow chromoprotein
12	6K_K1033910_2	6/17/2015 14:56:00	8.1	ng/µl	2.19	yellow chromoprotein
13	6M-K1033916-1	6/17/2015 15:13:00	13.6	ng/µl	2.28	lime chromoprotein

14	6M-K1033916-2	6/17/2015 15:15:00	12.7	ng/µl	2.2	lime chromoprotein
15	13L-E0040-1	6/17/2015 15:17:00	17.4	ng/µl	2.09	amp backbone
16	13L-E0040-2	6/17/2015 15:19:00	14	ng/µl	2.13	amp backbone
17	4N-E0020-1	6/17/2015 15:25:00	12.4	ng/µl	2.12	chloram. backbone

June 18th 2015

Objectives:

- Miniprep inoculated samples of chromoproteins
- Inoculate more chromoprotein colonies, 16E, 19E, 2I, 5F

Procedures:

- Standard TIAN gel miniprep kit
- Use SOC media for inoculation

Results:

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	260/280
1	6K	6/18/2015 11:57:00	28	ng/µl	1.99
2	13L	6/18/2015 11:58:00	57	ng/µl	1.97
3	6M	6/18/2015 11:59:00	45.1	ng/µl	1.93
4	4N	6/18/2015 12:01:00	42.2	ng/µl	1.97

June 19th 2015

Objectives:

- Electrophorese all mini prepped samples to check for proper purification
- Miniprep inoculated samples

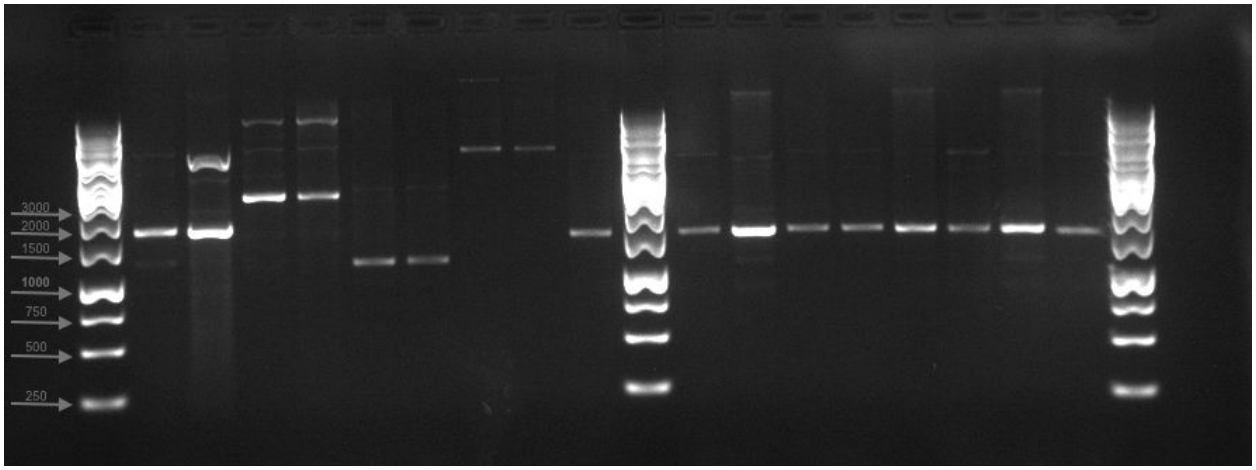
Procedure:

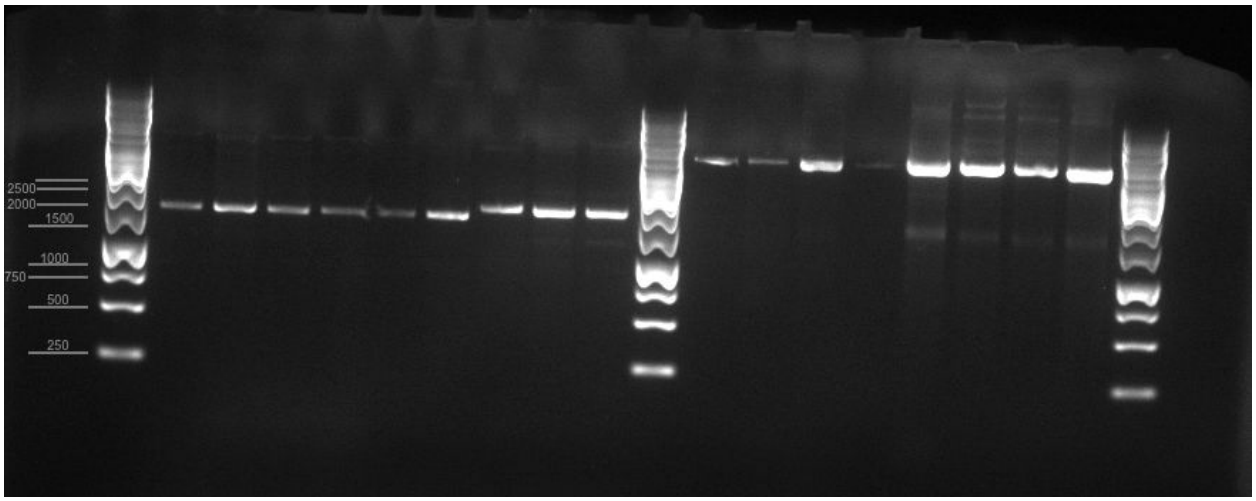
- Standard TIAN gel miniprep kit
- iGEM electrophoresis protocol

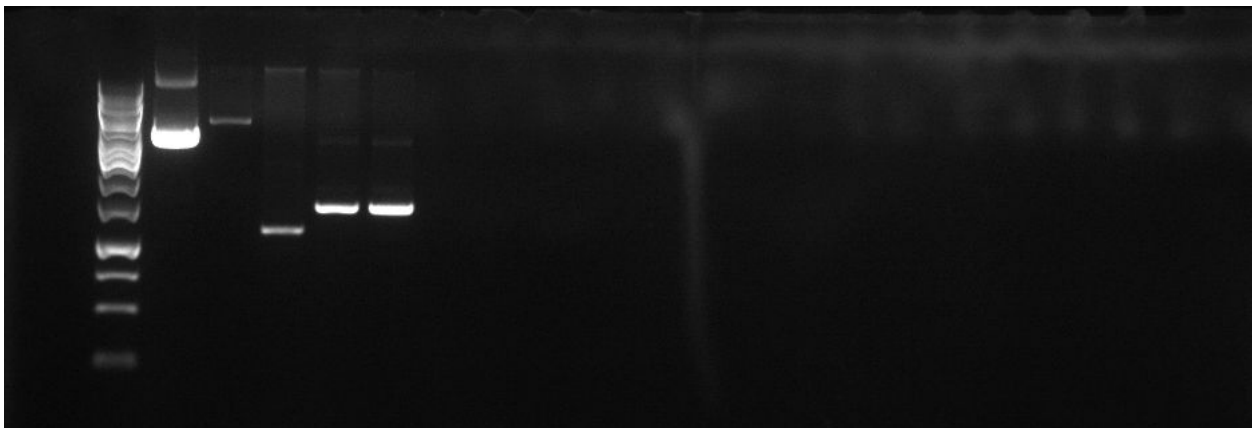
Results:

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	260/280
2	16E	6/19/2015 13:42:00	70.5	ng/µl	1.83
3	19E	6/19/2015 13:37:00	41.4	ng/µl	1.97
4	2I	6/19/2015 13:45:00	82.3	ng/µl	1.94
5	5F	6/19/2015 13:41:00	69.4	ng/µl	1.92

Electrophoresis Results







June 23rd 2015

Objectives:

- Run gels for plasmids
- Inoculate terminator for miniprep
- Transformation for orange luciferase and blue chromoprotein

Procedures:

Results:

June 24th 2015
Objectives:

- Miniprep terminator
- Inoculate all 10 plates of chromoprotein parts because of low yield

Procedures:
Results:

June 25 2015
Objectives:

- Miniprep chromoprotein parts 1-5
- Make competent cells

Procedures:

Results:

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	260/280
1	5F1	6/25/2015 13:21:00	93.8	ng/µl	1.91
2	5F_2	6/25/2015 13:23:00	100.1	ng/µl	1.95
3	2I_1	6/25/2015 13:24:00	58.7	ng/µl	1.92
4	2I_2	6/25/2015 13:25:00	105.4	ng/µl	1.96
5	1B_1	6/25/2015 13:30:00	71.2	ng/µl	1.92
6	1B_2	6/25/2015 13:31:00	50.8	ng/µl	1.95
7	19E_1	6/25/2015 13:33:00	121.3	ng/µl	1.94
8	19E_2	6/25/2015 13:34:00	91.5	ng/µl	1.96
10	6K_1	6/25/2015 13:36:00	59.9	ng/µl	1.9
12	6K_2	6/25/2015 13:53:00	74.7	ng/µl	1.91

June 26th 2015
Objectives:

- Miniprep remaining inoculated samples
- Make LB plates

June 29 2015

Objectives:

- Electrophoresis of purified plasmids
- Transformation of luciferase parts

The main goal today was to electrophorese all of the purified plasmids we are planning to use for the construction of the chromoproteins. From the results we got, we could see that we have some supercoiled DNA that would make the band of a certain plasmid seem much shorter that the actual length. Other than that, all of the plasmids seem to have been purified correctly with almost pure DNA. Not only that, we transformed all of the luciferases we are going to use as well as the lux operon, Renilla luciferase, and just for fun, an apple fragrance plasmid. We transformed these using our own competent cells from last week. There may be some problems with the bacteria giving our results from the efficiency transformation. Instead of the standard protocol, we used 13µl of cells per transformation because the cells are 8x more concentrated than the factory *E. coli*.

June 30th 2015

We harvested 2 colonies from each transformation from the day before and inoculated them in SOC culture. Because the yield of the colonies for the terminator we are using was slow, we are going to transform yet again. However because we are suspicious of our competent cells, we are also transforming the competent cells with the first RFP plasmid we mini prepped. Each transformation uses about 13µl of cells.

July 1st

Objectives:

- Transform RFP and CFP
- Inoculate luciferase samples

July 2nd 2015

Objectives:

- Transform competent using RFP and CFP with different conditions

July 3rd 2015

Objectives:

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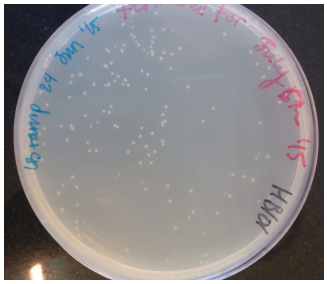
July 6th 2015

The first task this morning was to use the restriction digest products from last Thursday to ligate and transform HB101 cells. I used the iGEM ligation protocol, but quickly realized that the backbone I used was not really the backbone, but the ligation product of a different day. Therefore, we scrapped the new samples and moved on to inoculating the luciferase and company plasmids. I also inoculated 2 RFP colonies in case we needed a control plasmid. Finally, we transformed the terminator we are going to use for our ligations so that we have more of the plasmid to work with in the future.

Results

Inoculation: Predictably, after about 16 hours of incubation, the luciferases were not glowing neither under UV light, nor in complete darkness. After more research, the problems could be narrowed down to a number of things. 1) the SOC media was contaminated or , 2) The inoculation period in the shaking incubator was way too long, so long that the 12 hour lifespan of the light produced by the bacteria came and went, 3) The arabinose concentration in the culture was either too low or too high for the light to be produced. Looking in to the Cambridge 2010 iGEM group wiki, I found that the concentration they used to make the plates are grow the cultures were 100μM. We used a concentration of about 13320 μM, but the maximum light produced uses 10000μM. Therefore, I plan on making three new cultures of the green firefly luciferase to test. One will be using 100μM, another will have a concentration of 10000μM, and finally a culture that will have the original 13320μM concentration. If I am able, I want to grow two sets of three so that one set can grow from the time we are in lab (10:00 to 17:00), and another set that will grow overnight.

Transformation: The transformation of the terminator went extremely well. We used the HB101 competent cells our professor made herself because we ran out of the dH5alpha cells. Since this transformation is only going to be used to amplify the terminator plasmid, it is not a concern that we have used different cells.

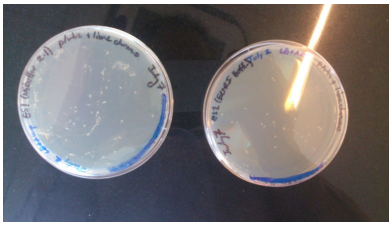


July 7th 2015

Today we are ligating together 2 combinations of 3 restriction digests that we have already digested. For the first ligation, we used 2μl of the linearized ampicillin backbone, 2μl of the promoter/rbs sequence digested with NEBuffer 2.1, and 2μl of the lime chromoprotein digest. The second ligation was 2μl of the linearized ampicillin backbone, 2μl of the promoter/rbs sequence digested with NEBuffer EcoRI, and 2μl of the lime chromoprotein digest. We are doing two because we want to test the efficiency of the NEBuffer 2.1 with the NEBuffer EcoRI. For the transformation, we are using 5μl of both products with 50μl of the HB101 cells to ensure that there is enough plasmid for a successful transformation. Also today, we mini prepped the terminator we inoculated the day before. We achieved results >100ng/μl for both samples.

Results

Transformation: Success!! The ligation product transformed and we had growth on the plates, both while using the NEBuffer 2.1 and NEBuffer EcoRI.



July 8th

Given the research about the arabinose concentrations, we made three different concentrations of arabinose, 13320μM, 10000μM, and 1000μM. The original concentration should have been 100μM, but the amount of arabinose needed was too small to make. We also mini prepped the luciferase cultures and ran a gel electrophoresis to check that we have pure DNA. We were going to transform them again with HB101 cells but we did not have enough time. Finally, we made cultures of the transformed ligation products to be checked in the morning.

Objectives

- Miniprep inoculated luciferase & do gel electrophoresis
- Innoculate the ligation samples Reida transformed yesterday

Procedure

- Miniprep with Biomiga
- Inoculation of ligation samples (in SOC broth)

Results:

- Miniprep Results:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	2I RFP 1	Student	7/8/2015 14:37:00	115.7	ng/μl	2.314	1.169	1.98	2.25	DNA	50
2	2I RFP 2	Student	7/8/2015 14:39:00	137.8	ng/μl	2.756	1.426	1.93	2.18	DNA	50
3	3J Renilla	Student	7/8/2015 14:39:00	74	ng/μl	1.48	0.763	1.94	1.81	DNA	50
4	2H Apple Fragrance	Student	7/8/2015 14:40:00	77.5	ng/μl	1.551	0.802	1.93	2.11	DNA	50
5	4J Orange Luc	Student	7/8/2015 14:41:00	139.7	ng/μl	2.794	1.429	1.96	2.21	DNA	50
6	2F Epic Luc	Student	7/8/2015 14:42:00	56.3	ng/μl	1.127	0.58	1.94	2.04	DNA	50
7	2H Green Luc	Student	7/8/2015 14:43:00	79.6	ng/μl	1.591	0.833	1.91	2.1	DNA	50
8	2J Yellow Luc	Student	7/8/2015 14:44:00	79.6	ng/μl	1.592	0.81	1.97	2.13	DNA	50
9	4L Lux Operon	Student	7/8/2015 14:44:00	77.5	ng/μl	1.549	0.809	1.92	2.17	DNA	50
10	2D Red Luc	Student	7/8/2015 14:45:00	261.7	ng/μl	5.233	2.654	1.97	2.26	DNA	50

- Gel electrophoresis results were not as expected.

Thursday July 9th 2015

Objectives:

- Miniprep the ligation products (inoculated overnight)
- Restriction Digest of the mini-prepped ligation products
- Transform luciferase (mini prepped yesterday)

Procedures:

- Use Biomiga protocol to miniprep the ligation products
- Standard transformation procedure

- Restriction digest using miniprep sample #4 (146.7ng/µL), E & S enzymes. Following NEB protocol

Results

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	EcoRI 1	Student	7/9/2015 13:10:00	145.3	ng/µl	2.905	1.399	2.08	2.25	DNA	50
2	EcoRI 2	Student	7/9/2015 13:11:00	102.8	ng/µl	2.056	1.007	2.04	2.18	DNA	50
3	EcoRI 3	Student	7/9/2015 13:12:00	68.5	ng/µl	1.371	0.7	1.96	1.98	DNA	50
4	Buffer 2.1 1	Student	7/9/2015 13:13:00	146.7	ng/µl	2.935	1.423	2.06	2.16	DNA	50
5	Buffer 2.1 2	Student	7/9/2015 13:13:00	113.6	ng/µl	2.272	1.109	2.05	2.13	DNA	50
6	Buffer 2.1 3	Student	7/9/2015 13:14:00	210.5	ng/µl	4.209	2.009	2.1	2.28	DNA	50

Nanodrop results of p/rbs + yellow/green chromoprotein digested with buffers NEB 2.1 and EcoRI

- Transformation results (viewed the next day)
  - All grew except YFP

July 13th 2015